AMENDMENTS TO THE SPECIFICATION

[0019] In a preferred embodiment, the X-ab conjugate is tested by streaking it onto a solid substrate, preferably a hydrophobic substrate such as a ~~PDVF~~ PVDF (polyvinyldifluoride) membrane, and more preferably a ζ-GRIP™ microarray chip (a non-conductive protein binding substrate) (Miragene, Inc., Irvine Ca.). After the X-ab streak has dried, conductance is measured using a voltmeter by applying a voltage across the streak. If the metal complex is present on the substrate a current will be conducted across the streak.

[0021] In accordance with one preferred aspect of the present biomolecular switch, the above-described X-ab and substrate can be used to distinguish diseased serum (comprising for example, the patient's antibodies against a disease pathogen, or a patient's autoantibodies in an autoimmune disorder) from healthy, non-immune serum (comprising for example, no specific antibodies against the disease pathogen or autoimmune antigen, etc.). The procedure involves spotting an antigen (e.g., a pathogenic antigen or an autoimmune antigen) onto a substrate, in accordance with known methods for preparing a protein microarray (see e.g., co-pending U.S. Pat. Appln. No. 10/376,351; incorporated in its entirety herein by reference thereto). In preferred embodiments of the present invention, the antigen is spotted onto a ζ-GRIP™ microarray chip. After blocking to minimize non-specific binding, the substrate is incubated with the test serum. Preferably, a parallel substrate, spotted with the antigen is blocked and incubated with a control (healthy) serum sample. After washing, the substrate containing any antigen-antibody complexes, is incubated with the X-ab solution, wherein the ab is specific for the patients antibodies (for example, an X-labeled anti-human IgG). The addressable antigen spots are analyzed for the presence of metal (ability to conduct an electric current) by passing a current across the spot between electrodes and measuring electrical conductivity, preferably using a digital multimeter.

[0022] In accordance with another preferred aspect of the present biomolecular switch, the above-described X-ab and substrate can be used as a highly sensitive digital detection means for detecting the presence of an analyte in a sample. In this variation, unlabeled antibodies ("ab_1"), which are specific to an epitope ("Ep_1") of a particular antigen ("Ag," e.g., anthrax protective antigen), are laid down on a substrate, e.g., preferably a streak on ζ-GRIP™. After blocking, an unknown sample comprising a mixture of possible antigens being screened for

the present of Ag is then contacted with the substrate comprising unlabeled ab_1. X-ab conjugate is then added. In one embodiment the ab portion of the X-ab conjugate is ab_1, which is specific for the same epitope Ep_1 on the Ag. In a variation, the ab portion of the X-ab conjugate is ab_2, which is specific for a second epitope Ep_2 on the Ag. The streak (or spot) is then analyzed for the presence of metal (ability to conduct an electric current) by passing a current across the streak (or spot) between electrodes and measuring electrical conductivity, preferably using a digital multimeter. Using a test cassette configured to accept the substrate slide between built-in electrodes, connected to a digital multimeter, which is connected to a data analysis/storage module, will allow nearly instantaneous testing for the presence of a particular antigen of interest.

[0023] In another embodiment, protein degradation can be monitored by the above-described system. Applicant has found that the level of specific X-ab binding to protein antigens decreases with time as a function of the extent of protein degradation. Thus, protein integrity can be readily tested in accordance with an embodiment of the present invention by laying down a solution containing the test protein in a streak or spot on a substrate, preferably ζ-GRIP™, and. then probing for epitope integrity by assessing the level of X-ab binding – by measuring conductance as detailed above, and in the specific examples below. Resistance will increase (conductivity will decrease) with degradation of the protein.

[0025] Preparation of metal-antibody (X-ab) conjugates – The following metal salts were used: Cadmium Chloride Anhydrous (hereafter referred to as $CdCl_2$), Sigma; Cobalt Chloride Hexahydrate (hereafter referred to as $CoCl_2$), Sigma: Cupric Sulfate Pentahydrate (hereafter referred to as $CuSO_4 \cdot 5H_2O$), Sigma; Nickel Chloride Hexahydrate (hereafter referred to as $NiCl_2 \cdot 6H_2O$), Sigma; and Iron(II) Sulfate Heptahydrate (hereafter referred to as $FeSO_4$), Aldrich. The antibody used was an anti-human IgG antibody labeled with alkaline phosphatase (hereafter referred to as γ-Human IgG-AP), Pierce Chemical. For the dialysis of X-ab, 10 x PBS without calcium or magnesium, BioWittaker (cat# 03367), and enzyme grade glycerol, FisherBiotech (cat # BP229-1) were utilized. The substrate used in these examples was ζ-GRIP™ microarray chips (Miragene, Inc., Irvine CA). To measure the conductance and current, a multimeter (BK Tool Kit 2707A), BK Precision was used in connection with a power supply (MW122A multi-voltage 2Amp DC Power Supply).

[0026] To test for diseased serum, SSA/Ro (Sjögren syndrome Type A) antigen, Immunovision, was used along with Casein in TBS Blocker, Pierce Chemical. SLE (systemic lupus erythematosus) patient serum (which has previously been tested positive for the SSA/Ro antigen) and control serum, pro-medDX, was also used. For parallel colorimetric quantification of the antigen-ab interaction, the BNCIP reagent (BCIP (chloro-3'-indolyphosphate p-toluidine salt) and NBT (nitro-blue tetrazolium C), Pierce Chemical) was used.

[0029] About 75-µl of the dialysed X-ab solution is streaked onto the ζ-GRIP™ microarray chips, where the solution is then allowed to dry. Banana clips (attached to some power supply and voltmeter) are attached to each end of the streak on the chip. Applying a 6V voltage from the power supply to the chip to the multimeter allows for the conductance and current across the chip to be measured. A schematic of this set up is shown in Figure 1. If the metal complex is present on the chip, the "circuit" closes, producing a current, which is representive of the X-ab conductance on the chip.

[0030] In order to test for diseased serum, a line of the SSA/Ro antigen was run across the ζ-GRIP™ microarray chip, the location marked (with a permanent marker), and the serum allowed to dry overnight. Then, the chips underwent a standard autoantigen assay: Once dried, the chips were incubated in blocker for 1-hr with agitation, followed by three washes in 1xPBS buffer (10-min each wash). About 100-µl of serum and 10-ml of 1xPBS is then used to incubate the chip for 1-hr with agitation. The chips are then washed three times in 1xPBS buffer (10-min each wash). Following, the Ni-ab solution is added to 10-ml of 1xPBS, so that the chips can then incubated in this solution for 1-hr with agitation. After washing three times with PBS, the chips are dried and the capacitance and current measured as described above. Colorimetrically, the line can be developed by incubating the chip in 10-ml of BNCIP reagent for 15-min, and stopping the reaction with ddH_2O.

[0032] The acquired data of duplicates of three different streaking condition on the ζ-GRIP™ microarray chips. $NiCl_2$-1 and $NiCl_2$-2 are two different chips, both streaked with $NiCl_2$ solution are shown in TABLE 1. C2-1 and C2-2 are two different chips, both streaked with anti-human IgG (AP) antibody solution only. N2-1 and N2-2 are two different chips, both streaked with the Nickel-anti-human IgG (AP) complex solution. Once dried, results were recorded in terms of each chips' current (µA), resistance (MΩ), and capacitance (nF).

[0037] FIG. 6 is a plot of the capacitance change of N2 and C2 streaked chips in a dry environment over a period of 26 days. Humidity was kept constant at 20%. The data in TABLE 1 gives the results of some preliminary work. For this experiment, six ζ-GRIP microarray chips were streaked. The first two ($NiCl_2$-1 and $NiCl_2$-2) were streaked with a 0.1% solution of $NiCl_2$. The next two chips (C2-1 and C2-2) were streaked with a 1:10 dilution of γ-Human IgG-AP in ddH_2O. And the final two chips (N2-1 and N2-2) were streaked with a solution of the Ni-ab complex. After drying and measuring, the results in TABLE 1 were obtained. As can be seen, the $NiCl_2$ solution does not conduct. This is due to the hydrophobicity of the solution, as the ζ-GRIP™ microarray chip will only bind hydrophilic substances. The C2 chips do not conduct as well compared to N2. The signal for N2 is about four to eight times greater than C2 in terms of current and about ten times greater in terms of capacitance.

[0040] For FIG.'s 2 and 3, there is an apparent peak at ~ 5 μl/ml of antibody. There is no signal at 0 μl/ml antibody, as charged nickel does not bind to the ζ-GRIP™ chip. Nickel will only be apparent if the γ-Human IgG-AP antibody is bound to it. Still, the experiment was repeated for the range of 0 μl/ml to 20 μl/ml antibody, with samples streaked in increments of 2 μl/ml, so as to find the actual peak location. FIG.'s 4 and 5 illustrate the plots of these results. FIG. 4 is a plot of the capacitance of nickel anti-human IgG (AP) conjugates as a function of the amount of antibody for four different percentages of nickel (0%, 0.1%, 0.5%, and 1%), where FIG. 5 plots current for the same sample rather than capacitance. Each sample is streaked three times, where each streak is measured three times. Plotted data is the average of these results.